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THE STRATIGRAPHIC DISTRIBUTION OF DRY MATTER AND NITROGEN IN CATTLE HIDES*

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ABSTRACT

A precision slicing technique was employed to cut consecutive slices of equal thickness parallel to the grain surface of a cattlehide. These slices were analyzed to determine the quantitative distribution of various components through the thickness of the hide. A dehydration procedure was developed which enabled the contents of these slices to be calculated on a dry-weight basis. The dry-weight yield and the nitrogen content of the slices revealed marked differences in composition through the thickness of the hide. The flesh and grain areas contained less dry matter than the corium. A similar trend was also evident from the nitrogen values.

INTRODUCTION

The various components of hides and skins and their changes during leather manufacture have been the object of many studies (1). However, extensive application of technology to the problems of the leather industry keeps demanding a sharper and more detailed description of the true nature and structure of hides. Histological studies have supplied detailed descriptions of the location of the organized structures of hides and some information on the occurrence of the unorganized constituents. Chemical analysis, however, has been applied chiefly to the determination of the gross amount of constituent present in the whole thickness of a hide. There are only a few recorded attempts to apply chemical analysis to determine structural features in hides or leathers.

As early as 1922 Balderston (2) attempted to obtain greater detail on the distribution of grease in leathers by splitting the leather into seven layers and analyzing each layer separately. An abbreviation of this technique—the analysis of the grain, middle, and flesh splits—was applied quite thoroughly

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to the analysis of fats and chromium in leathers. References to these studies were presented by Kritzinger and Theis (3), who utilized the precision of the microtome to slice leather specimens into exactly ten equal layers parallel to the grain surface. Their studies of the stratigraphic distribution of chromium content in leathers gave direct quantitative data to clarify many aspects of this important problem.

This stratigraphic analysis technique employing the microtome was used directly on fresh hide material by Mellon and Korn (4) to demonstrate the network structure of elastin in the grain area of cattlehides. This ability of stratigraphic chemical analysis to supplement and elaborate upon information obtained by histological studies encouraged its application to other chemical determinations on raw hides.

EXPERIMENTAL

Preparation of hide.—A freshly flayed steerhide was washed with cold water for one-half hour in a slotted drum to remove blood and debris. After fleshing, an area about 12″ x 18″ was cut from the middle of the bend adjacent to the backbone. This piece was stored at −20°F. for a minimum of six months to assure that all rapid changes due to freezing had occurred.

For each determination a piece about 3″ square was removed from this piece, thawed, and shaved with a dry razor to remove the hair level with the epidermis. The shaved piece was kept in cold water overnight with 0.015% phenylmercuric acetate as a preservative. This assured an even hydration of the entire sample. In the morning the shaved piece was cut into approximately 1.5-cm. squares using a sharp dissecting knife. These pieces were kept immersed in water until removed for freezing. The pieces with sides most nearly perpendicular to the grain surface were chosen for slicing. Twelve pieces were used for each run. To facilitate the production of truly flat grain surface, these were placed against a smooth aluminum sheet resting on pieces of dry ice. A flat-surfaced glass plug weighing 57 g. was placed on the flesh side to press the grain uniformly against the plate. When the piece was frozen to within a few millimeters of the flesh side, it was removed from the plate and frozen flesh side down on the microtome stage. A layer of ice several millimeters thick under the piece kept the microtome blade from hitting the stage when the last slices were cut.

Sectioning.—The stage was adjusted so that the grain surface was approximately parallel to the knife path. Slices were cut 0.1 mm. thick as shown in Fig. 1. If a cut across the entire piece was not obtained by the fourth slice, the piece was rejected and a new piece substituted. The cut slices were put immediately into small beakers containing about 10 ml. of acetone. The five slices which comprise each layer were collected in the same beaker. After the entire piece was successfully sliced, the slices were

transferred to 50-ml. round-bottomed centrifuge tubes each containing 25 ml. of acetone.

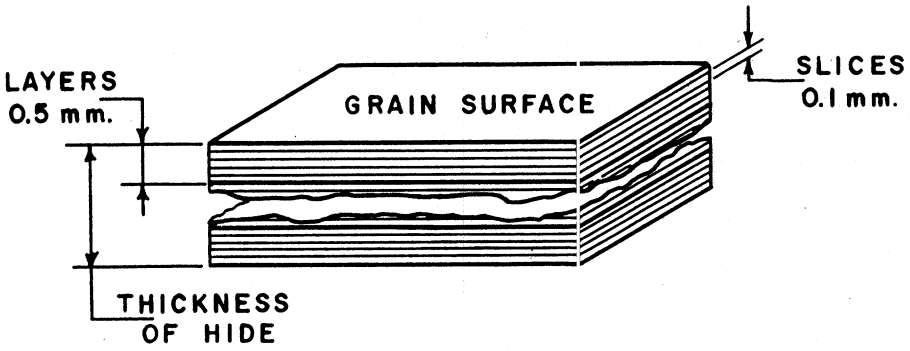

FIGURE 1.—Details of stratigraphic slicing method.

After the 12 pieces were sliced, each centrifuge tube except the last contained 60 slices all taken from the same layer at a fixed distance from the grain surface. The last tube contained between 60 to 120 slices and was called the residue. All the pieces did not give an identical number of slices, and to reduce the number of analyses the last partial tube of slices was added to the previous tube. Twelve or thirteen complete layers plus the residue were obtained for each run.

Dry weight determination.—After 18 hours the acetone was decanted, and fresh acetone was put on the slices. Eight hours later this acetone was decanted, and the slices still in the centrifuge tubes were dried in a vacuum oven for 16 hours at 45 °C. The tubes were weighed and then redried in the vacuum oven for an additional 8 hours to assure a constant dry weight. A stream of air, dried with magnesium perchlorate, flowed through the oven to sweep out liberated moisture. The increase in weight of the tubes over their tare weight was reported as the dry weight of the layers. The data for four such experiments are presented in Table I.

Nitrogen determination.—The dry slices were suspended in 20 ml. of 6*N* hydrochloric acid and refluxed for 16 hours. The hydrolyzate was diluted to 100 ml. and 2.0-ml. samples were removed for the Kjeldahl nitrogen determination. The digestion mixture was 2 ml. of sulfuric acid, 1.20 g. of potassium sulfate, and 0.03 g. of mercuric oxide. Digestion time was two hours from clearing. The ammonia liberated from the digestion mixture was caught in boric acid and titrated with standard acid.

The data for four such experiments are presented in Table I.

TABLE I

THE DRY WEIGHT AND NITROGEN CONTENTS OF LAYERS OF ACETONE-EXTRACTED STEERHIDE

(Milligrams per layer)

Layers*	Run 1		Run 2		Run 3		Run 4	
	Wt.	N	Wt.	N	Wt.	N	Wt.	N
1	387	62	278	46	269	44	150	25
2	435	72	324	55	366	60	267	44
3	522	90	391	66	366	61	306	52
4	712	125	537	91	576	99	463	80
5	812	144	722	129	647	110	516	90
6	941	166	718	129	687	124	577	100
7	911	160	761	132	640	117	567	99
8	1096	191	760	134	693	120	531	91
9	923	164	869	154	692	121	564	98
10	917	161	707	125	597	103	576	98
11	912	158	676	124	676	116	609	104
12	787	122	712	125	562	98	567	99
13	662	113	564	98	†	†	367	60
R‡	999	169	759	129	1.039	170	798	136

*Numbered from hair surface.
†This specimen was one layer thinner.
‡The residue which includes the last full layer plus the remaining partial layer.

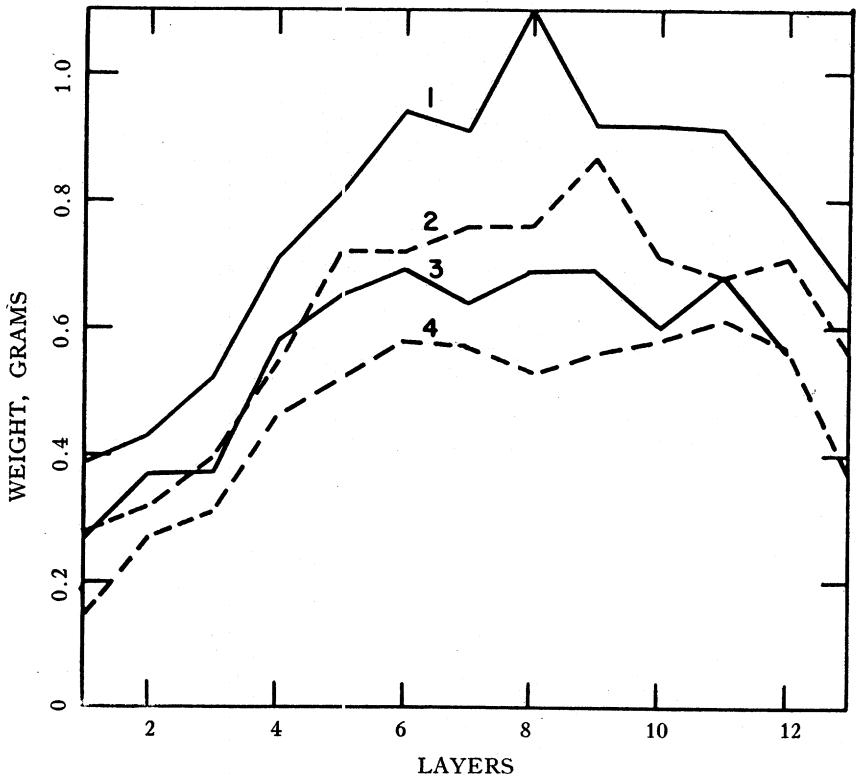

FIGURE 2.—Distribut on of dry weight in acetone-extracted hide.

DISCUSSION

The curves of Figs. 2 and 3, which represent the distribution of the dry matter of a steerhide, show a pronounced inflection at the fourth layer from the grain. The decrease of hair root residues is quite marked within the fourth layer, and very few hair roots are visible in the slices of the fifth layer. This indicates that the fourth layer is also the transition between the grain and corium layers. Therefore, it is apparent that the concentration of dry matter in the corium is approximately twice the concentration in the grain area. This is the reverse of the picture presented by the fibrous structure, for this shows a coarse network of coarse fibers in the corium and a dense network of fine fibers in the grain area.

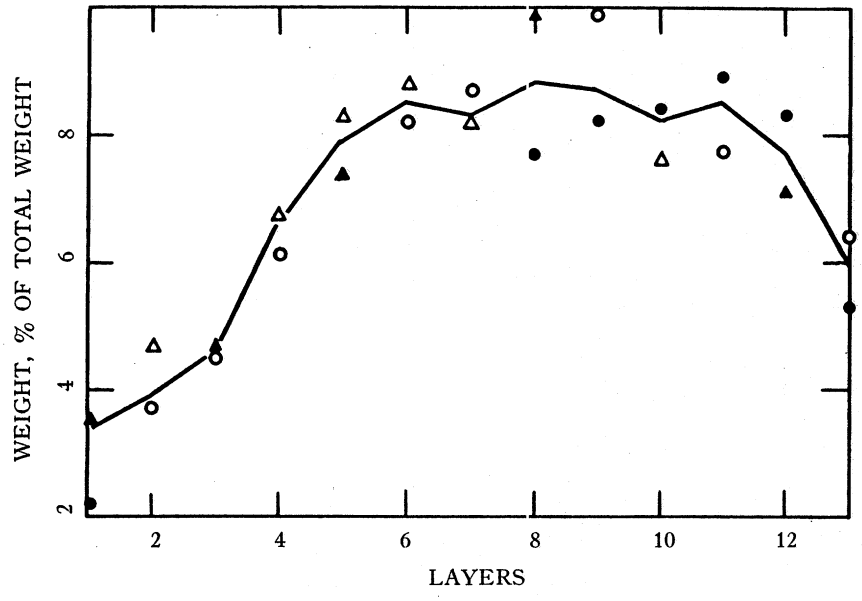

FIGURE 3.—Distribution of dry weight as percent of total dry weight: median line and extreme points.

▲ run 1, △ run 2, ○ run 3, ● run 4.

It is unfortunate that the necessity of embedding the hide specimens in ice to obtain satisfactory slicing on the microtome prevented the determination of the original weight or moisture content of the layers, for this may have supplied some interesting observations. The use of acetone to dehydrate the tissue and of a vacuum oven with a flowing dry air stream to obtain complete removal of acetone and residual moisture at low temperature gave a comparable dry weight without producing serious changes in the nature of the carbohydrates and proteins. This technique removed some free fatty ma-

terial from the layers, but this fat did not amount to more than 12% of the dry weight even in the layer containing the sebaceous glands. Therefore, the loss in dry matter due to the extraction of fat by the acetone can explain only a small portion of the difference between the grain and the corium. The data, however, should be considered as those from an acetone-extracted frozen hide rather than for a freshly flayed hide, although there is probably little difference between the two as far as the dry matter and nitrogen content are concerned.

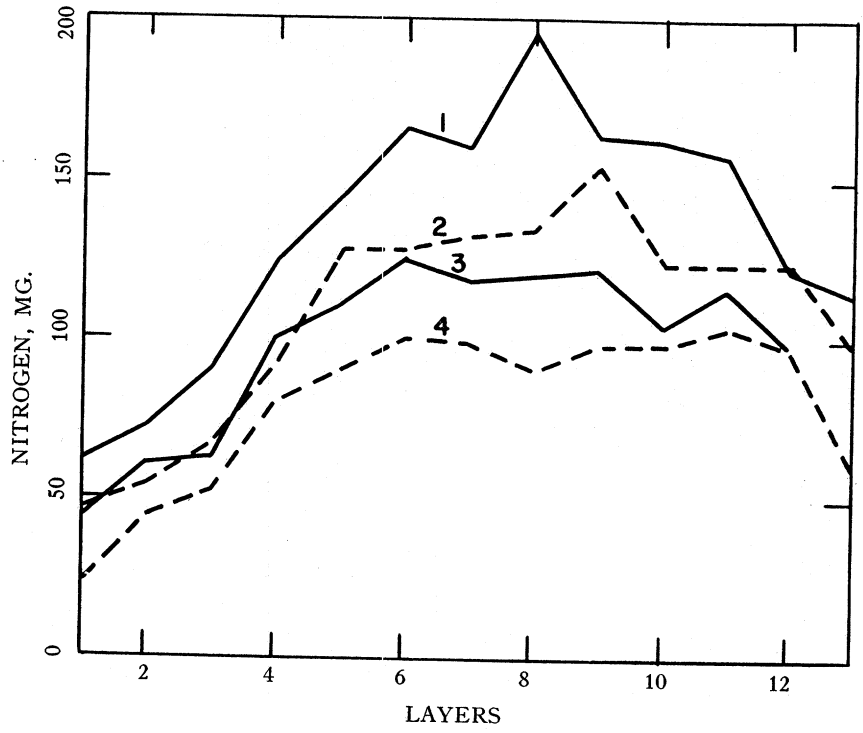

FIGURE 4.—Distribution of nitrogen in acetone-extracted hide.

The dry-weight data for four runs are presented in Fig. 2. The curves do not fall together because different amounts of hide were sliced for each run. The dry weight of the first layer appears erratic because the first few slices cannot be cut uniform in area; thus the first layer represents a volume of tissue different from the subsequent layers of the same run. The remaining slices, however, have a uniform area and thickness and represent a definite volume of hide tissue.

In addition to the pronounced inflection in the curves at the grain-corium boundary there is a definite downward trend of the dry-matter content curve

on the flesh side of the hide. This change does not appear to be as pronounced as that on the grain side and this may be partly due to the omission from the plot of the residual layer from the flesh side. This residue was not included in the figure because it represents a different volume of tissue from the other layers and is not directly comparable with them.

The reproducibility of the method can probably be judged best if the four runs are put onto a comparable basis. This is easily done by plotting the weight of each layer as a percentage of the total dry weight for all the layers including the residue. The curves would crisscross indistinguishably, so Fig. 3 presents only the curve for the median value for the four runs and the upper and lower extreme points of the four runs. It is clear that a nearly random distribution of values is obtained around the median line and that the variation of the values is much less than the observed difference between the grain, corium, and flesh regions of the hide.

Since the slicing technique provides equal volumes of tissue for each layer, and since the density of the fibrous elements which make up the bulk of these layers do not differ greatly, the nonfibrous components of the hide may be responsible for the difference in concentration of dry matter between the

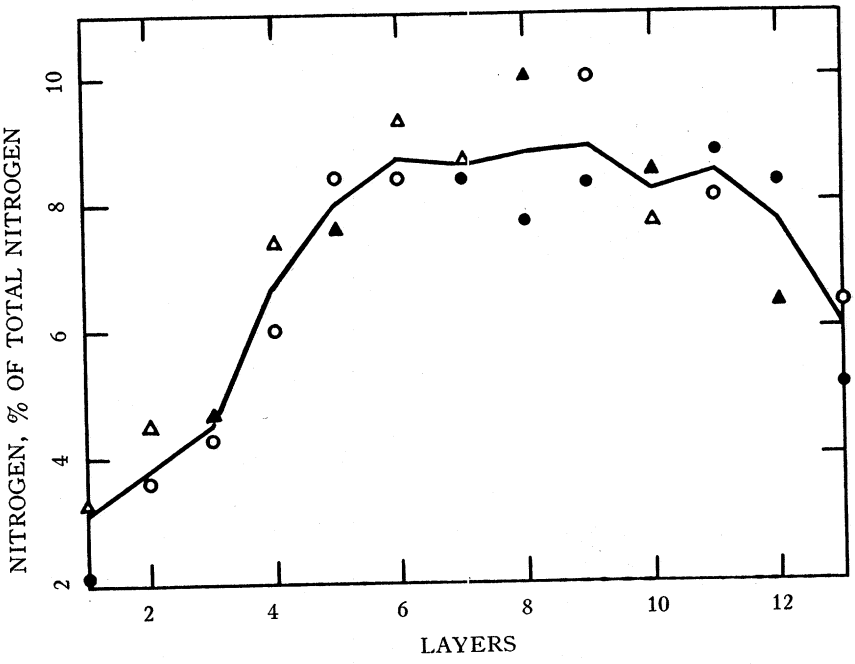

FIGURE 5.—Distribution of nitrogen as percent of total nitrogen: median line and extreme points.

▲ run 1, △ run 2, ○ run 3, ● run 4.

grain and corium layers and between the flesh and corium layers. Histological evidence shows that the mucopolysaccharide-type materials are more pronounced in the grain and flesh layers of a hide. These materials form gels between the fibers, and these gels can hold enormous amounts of water for their dry-matter content. If this proves to be the true explanation, the grain region of a hide would contain more water than has previously been indicated.

Another, though less plausible, explanation is that the hair follicles, although filled with the hair shaft, still contain a large amount of void space. Both of these possibilities are now being explored.

The nitrogen-content values which are shown in Fig. 4 are almost superimposable on the dry-weight-content curves of Fig. 2, and when these data are expressed as percentages of the total nitrogen in the entire thickness of the hide, the median curve shown in Fig. 5 is again almost superimposable on the dry-weight median curve shown in Fig. 3. The distribution of the extreme points indicates that no one run is particularly out of agreement with the median curve.

This marked similarity between the nitrogen and dry-weight curves emphasizes that the dry matter of a hide is predominantly proteinaceous in character. However, when the nitrogen values are calculated as a percentage

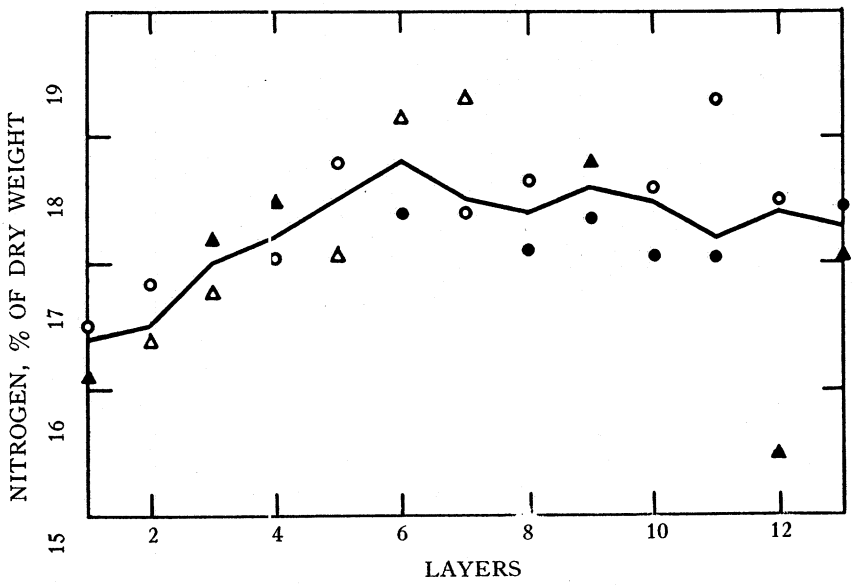

FIGURE 6.—Nitrogen content of slices on a dry-weight basis: median line and extreme points.

▲ run 1, △ run 2, ○ run 3, ● run 4.

of the dry weight for each layer, they show (Fig. 6) that the nitrogen content of the dry material in the grain layers is lower than that in the corium layers. This indicates that more material either lacking or low in nitrogen content is present in the grain than in the corium. This is one of the reasons why most of the purified hide collagens are made from corium splits of the hide.

The pretanning treatments usually remove most of the noncollagenous material from the hides. Therefore, it would make little difference whether the decreased concentration of dry matter in the grain were due to voids along the hair shaft or moisture absorbed in the mucopolysaccharide gels. The effect as far as finished leather is concerned would be that there is considerably more space around the fibers in the grain and flesh regions than there would be in the corium region.

Gustavson (5) has claimed that the degree of swelling of the hide substrate at the moment of tanning is the governing factor for the distribution of chrome compounds within the leather. Since the chromium content of the grain layer has been found by many workers (3) to be higher than that of the corium, Gustavson felt that alterations of the grain membrane by swelling must be more deep-going than changes in the flesh section. This could easily be possible if the fibers of the grain area had less spatial restriction to swelling than the fibers of the corium, and this possibility is clearly indicated by the data of the present paper.

Furthermore, Theis and Weidner (6) showed that the use of salt in the chrome liquor could level out the chrome concentration if the acid concentration were correct. They did not consider the swelling effect at that time, but later Theis (7) agreed with Gustavson that the swelling factor must be considered. This would argue that salt has a different effect upon the swelling of collagen fibers in the grain and in the corium. The present study would argue that the effect of salt is to reduce the swelling of the collagen fibers in the grain to the same degree of swelling as the corium fibers, which are restrained from swelling to their maximum degree by their compact environment.

The probability of a more open fibrous structure in the grain area than elsewhere in the hide may revolutionize present concepts on the treatment of the grain. Many grain defects such as break, cracking, and scuffing may be enhanced by a lack of appreciation of the natural porosity of the grain region of the hide.

CONCLUSION

The grain region of a hide appears to have a considerably more open fibrous structure than previously believed, for the dry matter per unit volume is approximately only half that of the corium. The flesh region also appears to be more open although to a less degree than the grain. These facts sub-

stantiate some theories on the effect of salt in leveling the distributic chromium in chrome-tanned leathers.

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DISCUSSION

DR. R. M. LOLLAR (Tanners' Council Research Laboratory, Universit Cincinnati): It is certainly interesting to see this paper, which empha the difference in the characteristics of the grain of skins and hides in com son with the characteristics of the corium. There are a couple of quest that I would like to ask Dr. Mellon. First, does he have any informatio the relative extent to which the acetone dehydration removed the fat? the removal of the fat complete or not?

DR. MELLON: I do not believe that the removal of fat was complete. have made some studies along that line, and our main difficulty is interpre the data, since there is such a wide variation of fat in a hide. You can practically any area of the hide, cut out an inch-square area with a determine the amount of fat by hydrolyzing the sample and extracting petroleum ether, and you may find a twofold difference in the fat con between two adjacent one-inch squares. We have done that any numb times. For this reason you cannot do a slicing experiment as we have here and expect to make a fat study that gives reasonable results. We h in the early stages of our work, determined the fat that was extracted in acetone. In no case was it more than 12% of the total dry weight, and was in the layer where the sebaceous glands exist.

DR. LOLLAR: One other question: Is it perhaps a reasonable interpr tion of the increase in the nitrogen content on the dry basis, presented in of the later slides, to assume that that is primarily reflecting collagen pur

DR. MELLON: I think there are a few people here in the audience— Cassel for one. . .and Dr. Kanagy—who have made purified collagens believe Dr. Lollar here has been in on that work, too. All of us who h

made purified collagens at one time or another know that we usually try to take our material for making collagen out of the center of the corium. That is because, as these results indicate, there is much less other nitrogenous material and much less carbohydrate material in the center of the corium, and we feel that the fewer the impurities you have to start with, the better chance you have of obtaining a pure material.

DR. HENRY B. MERRILL (B. D. Eisendrath Tanning Company): While it is true that as chrome tanning is ordinarily carried out you do have more chrome fixed in the grain and in the flesh than in the center of the skin, that depends on how the stock has been treated before it is tanned, how it is neutralized after it is tanned, and what kind of chrome liquor is used. By varying these factors you may get much greater fixation of chrome in the center, or much greater fixation in the grain and flesh, or practically a uniform fixation throughout the whole thickness of the skin. I believe those factors have far more to do with the distribution of chromium than any differences in the structure or composition of the hide itself.

DR. MELLON: Any of those differences require some difference between the grain, the flesh, and the corium areas. You give the same treatment to the same fibers; you have your hide in the same vat. There must be some difference. We all claim here that our tannin penetrates all through the hide—but how are we going to explain those differences? We make a difference in our tannin solution which causes a difference in pickup by the different sections of the hide—the grain, the corium, and the flesh. We are trying to draw out the fundamental facts which might explain these differences, and that is the object of our paper: to try and find some fundamental facts. We are submitting these to see if we cannot explain some of these phenomena.

DR. MERRILL: The point is that during actual tanning you do *not* treat all portions of the skin—all layers of the skin—in the same way. In pickling, you frequently acidify only the surface and leave the center much less acid or actually alkaline, and the same thing in reverse happens when you neutralize after tanning. You neutralize the grain and not the center. So you do not treat the different layers of the skin in the same way.

DR. J. R. KANAGY (National Bureau of Standards): Our work on impregnation with polymers would certainly bear out the work that Dr. Mellon has done. We always find that there is much more of the polymer in the grain than in the flesh or the corium. As a matter of fact, if you use material of too large molecular weight, it will all be in the grain or in the flesh, and there will be none in the corium where you actually want to put the material. For that reason you have to use a very low-molecular-weight polymer in

order to impregnate it properly. The work which has been done at the Bureau on pore sizes by use of the mercury porosimeter also agrees with Dr. Mellon's results this morning, indicating that the porosity of the grain and of the flesh is much greater than that of the corium.

DR. H. G. TURLEY (Rohm & Haas Company): I would like to say, in disagreement with Dr. Mellon, that it is not surprising that the grain is less dense than the corium, even histologically. If you look at a correctly prepared section under the microscope, you will see many empty spaces in the grain area. The hair does not fit the follicles perfectly, in the sweat glands there are holes, and so on. We have to realize that in the grain we have an active part of the skin. It is changing all the time. There is loosening of the hair and epidermis and so on. There seems to be in nature a certain elasticity or looseness to the grain of a skin. It stretches more than the underlying part, and some of the phenomena such as "goose pimples", for example, are associated with the grain layer only, being pulled up by the *erector pili* muscles. It would be a most horrifying effect if the whole skin were to swell up! I am sure practical tanners agree that one of the difficulties of leathermaking is to make what, according to your diagram, is about a 40% amount of solid matter of the grain equal finally to the 100% solid matter of the corium. So that the tanner is always plagued with the grain being loose (particularly in shoe leather), giving him poor break and other troubles.

DR. LOLLAR: Dr. Turley has certainly emphasized the non-histological difference of the grain area of the skin in comparison with the corium, and I must agree that the dissimilarity in density is not surprising.